Environmental Health Perspectives
Vol. 16, pp. 147-156, 1976

Secretory Cells of the Peripheral Pulmonary Epithelium as Targets for Toxic Agents

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The extracellular lining of the pulmonary peripheral airways is of vital importance to the lung. **In this report, some aspects of the pulmonary extracellular lining and the epithelial cells believed to be responsible for its formation and secretion have been briefly reviewed. The influence of a number of toxic agents on the extracellular lining either directly or via those cells involved in its formation indicates that the extracellular lining may be important in understanding numerous toxic agent interactions with the lung.**

The peripheral airways of the lung, that is, the bronchioles and alveoli, are lined with an extracellular layer of material (*1, 2*) which is essential for the prevention of atelectasis. Under conditions where the extracellular layer is absent, reduced, or altered, breathing is impaired (3-8). The nature of the extracellular lining is complex, consisting primarilyof polar lipids and proteins as well as other materials such as water, inorganic ions, and some low molecular weight organic molecules. The lipid components have received the greatest attention from researchers, although recently a number of the protein constitutents have been identified. The origins of all the known components of the extracellular layer have not been elucidated; however, the secretory cells of the peripheral airways have been implicated in the production of some of the constituents. In view of the essential nature of the extracellular lining, the cells and processes responsible for its production would appear to be of great importance to the lung and consequently should be of considerable concern to the toxicologist. The secretory cells of the peripheral airways could be key sites through which many inhaled environmental agents, both gaseous and particulate, exert their effects upon the lungs.

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In the discussion that follows, we will describe some salient features of the peripheral airways and the cells thought to be responsible for the formation of some of the constituents of the extracellular lining. In addition, we will focus attention upon the response of this system to toxic agents.

The Extracellular Lining

All of the branches of the upper and lower pulmonary airways have an extracellular lining. The epithelium of the upper airways, including the bronchi and trachea, is covered with a mucous layer. The mucins that compose this layer are thought to be secreted by goblet cells (Fig. 1) that are part of the pseudostratified columnar epithelium. The abundant cilia of this epithelium beat continuously in such a manner as to move the mucous layer, along with free cells and small particulates, up the respiratory tract. This "mucociliary escalator" begins in the regions of the respiratory bronchioles. The response of the respiratory mucociliary system to irritants has been previously reviewed (*9, 10*). The lower airways consisting of bronchioles and alveoli do not contain mucin-producing cells in their epithelium, and consequently the extracellular lining is quite different in composition from that of the upper airways. Although the extracellular lining of the lower airways may also be involved in the trans-

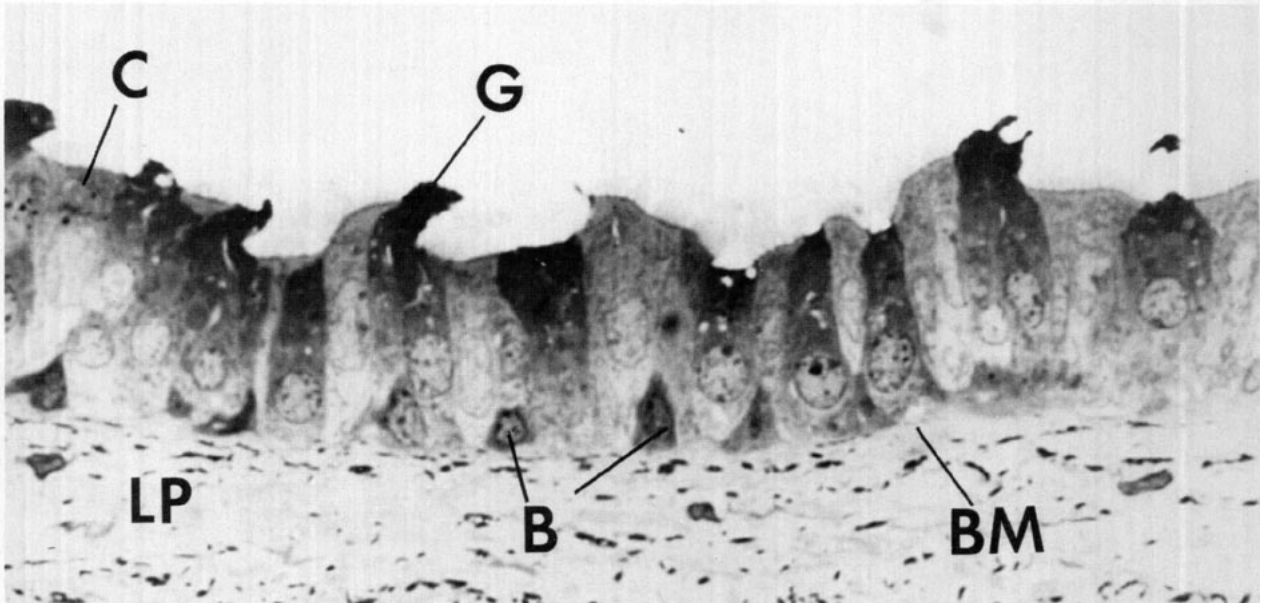

FIGURE 1. Longitudinal section of rabbit bronchial epithelium with part of the lamina propria (LP). The Epon-embedded section was stained with toluidine blue and shows the pseudo-stratified columnar epithelium common to the upper airways. Cells are attached to a basement membrane (BM). The basal cells (B) divide and apparently differentiate to form the ciliated cells (C) and the mucus-secreting goblet cells (G). The apocrinic secretion process of the goblet cells is represented by the intense staining of the apical region of these cells. Typical histological patterns of the tracheo-bronchial epithelium in response to irritant gases or particulates are loss of cilia, basal cell hyperplasia, and excessive goblet cell secretion. (Magnification ×1280).

port of particulate materials from the lung, its primary function appears to be the stabilization of the alveoli and bronchioles. The extracellular lining appears to prevent the collapse of the peripheral airways by reducing surface tension at the air-cell interface at low lung volumes. The surfactant properties of the extracellular lining have been reviewed extensively (*11-14*).

Electron micrographs of the extracellular lining overlying sections of alveolar type I cells are shown in Figure 2. Preservation of the lining is not easily achieved during fixation of the lung for electron microscopy and perfusion of the fixative through the vasculature is essential (*2*). Generally, the lining appears amorphous and granular, although its appearance seems to depend upon the fixation procedure and method of staining used for its visualization under the electron microscope. Other electron micrographs have been published in which a thin superficial osmiophilic layer extends over an amorphous subphase (*2, 15*). The phospholipid-containing latticelike structures known as tubular myelin (*16*) seen at the surface of the extracellular lining in Figure 2, are often found in the alveoli. Their function within the lining is not known.

The extracellular lining of the peripheral airways is generally sampled by lavaging the lungs with isotonic saline or a balanced salt solution *via* the trachea. This process removes extracellular material from both upper and lower airways; consequently, interpretation of analyses made on the lavage effluent must be made with caution. For example, while it appears that most of the phospholipid components of the lavage effluent arise from the extracellular lining of the peripheral airways, some minor components could arise from the upper airways and their origin would be mistakenly attributed to the lower airways. In addition to extracellular components of the lining, the lavage effluent contains alveolar macrophages and other mononuclear cells. The cells can be readily removed from the lavage effluent by differential centrifugation.

Compositional analyses of the cell-free lavage effluents vary considerably although most reports agree that the major surface-active component and the major phospholipid of the extracellular lining is dipalmitoyl lecithin (*17*). Many other minor lipid components (*18-20*) are also found in pulmonary lavage effluents. The major phospholipid components appear to be secreted by the alveolar type II cell although considerable controversy has been associated with this point of view. The controversy concerning the cellular origins of pulmonary surfactant was initiated by

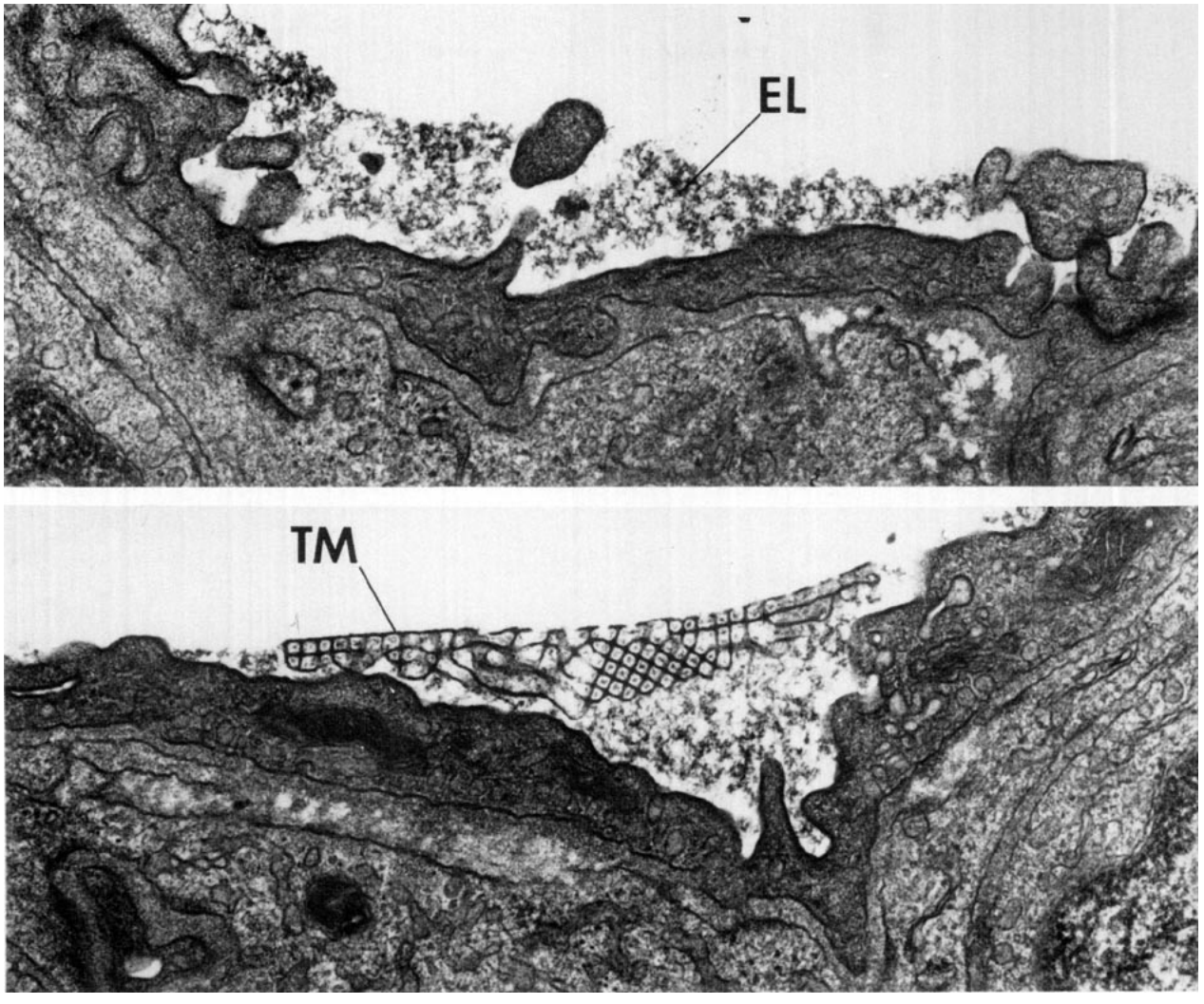

FIGURE 2. Extracellular lining material (EL) overlying alveolar type I cells (rabbit). Tubular myelin (TM) figures are often found in the extracellular lining of the alveoli. (Magnification × 61,560).

the observations of Niden (*21*) that the bronchiolar Clara cell may be secreting phospholipids and that the type II cell might be involved in the subsequent removal of phospholipids from the extracellular lining via a phagocytic process. Later studies have been published purporting secretion of surfactant phospholipids by the Clara cell (*22, 23*) as well as phagocytic activity of the type II cells (*24-26*). However, many other studies have been made which neither support phospholipid secretion by the Clara cell nor phagocytic activity for the type II cell.

The major protein component of pulmonary lavage effluents is albumin which has been shown, using immunochemical methods, to be a normal constituent of the extracellular lining (*27*). Similar immunochemical methods have been used to show the presence of IgG at the lining (*28*). Both albumin and IgG are probably found in the respiratory tract as a result of transudation of serum proteins from pulmonary capillaries. Secretory IgA, which appears to have an important antibacterial and antiviral function at the mucous surface of the distal lung is reported to be synthesized locally by plasma cells in the lung lamina propia or near glandular epithelial ducts (*29, 30*). Also, the IgA found in secretions differs from serum IgA in molecular weight and composition. Other factors may be present in the extracellular lining which assist in the protection of the

lung against certain bacteria. The bactericidal activity of alveolar macrophages against *S. aureus* appears to be stimulated in the presence of alveolar lining material (*31*).

The Clara Cell

The nonciliated Clara cell (Fig. 3) is one of two major cell types found within the bronchiolar epithelium. The other major cell type is the ciliated cell. The nucleus of the Clara cell is located toward the base of the cell and the cytoplasm bulges into the lumen of the bronchiole. A distinctive feature of the rabbit Clara cell is the abundance of smooth endoplasmic reticulum; rough endoplasmic reticulum is very sparse. These cells generally possess prominent Golgi and numerous mitochondria. As with the murine lung Clara cell, two types of mitochondria may be observed in Clara cells from the rabbit. Many mitochondria appear large and spherical with small and infrequent cristae, while others appear smaller and elongated with numerous cristae.

The functions of the Clara cell within the bronchioles of the lung are, at present, poorly understood, but considerable emphasis has been placed upon its role as a secretory cell. Morphological characteristics of the Clara cell indicate that the cell is secretory but the nature of its secretions and the manner in which secretion occurs has yet to be unequivocally determined. Both merocrinic and apocrinic secretory processes have been described. The classic studies, published in 1937, of Clara (*32*), after whom the nonciliated bronchiolar epithelial cell has come to be named, suggested apocrinic secretion as a possible process through which the Clara cell may contribute materials to the extracellular lining of the peripheral airways. Since then other investigators, using more advanced microscopic techniques, have reported similar observations (*21-23, 33, 34*). The secretions have been described as mucoid (*34*), nonmucoid (*32*), and surfactant (*21, 22*).

Merocrinic secretion of the cytoplasmic granules by the Clara cell has been considered a possible, if rarely observed, event (*35, 36*). The granules appearing as amorphous electron dense bodies are often found concentrated in apical regions of the Clara cell. The contents of these cytoplasmic granules are not known but histochemical studies indicate the presence of protein and nonextractable phospholipid (*35*). Unlike the lamellar bodies of the type II cell, the contents of the Clara cell granules are not extractable by chloroform-methanol mixtures and consequently are unlikely to be a storage site for saturated phospholipids of the extracellular lining (*37*).

The Type II Cell

In view of the large body of accumulated evidence, there seems little doubt that the type II cell is secretory. The type II cell is attached to the basement membrane and is one of two major epithelial cells located within the alveolus. The type II cell often assumes a somewhat cuboidal appearance and is easily distinguished from the type I cell, whose highly attenuated cytoplasm is spread over most of the basement membrane.

Unlike many other secretory cells, the Golgi apparatus of the type II cell is not overly prominent. The cytoplasm also contains mitochondria, endoplasmic reticulum, and small vacuoles, none of which lend morphological distinction to the cell. Numerous microvilli are found on the free surface of the cell and tight junctions exist where the type II cell is in contact with type I cells. The most distinctive feature of the type II cell is the cytoplasmic concentric lamellar organelles which are usually referred to as lamellar bodies (Fig. 4). Lamellar bodies vary in size from approximately 0.25 to 3 μ in diameter in the rabbit, and each one is enclosed by a limiting unit membrane. The bodies contain an osmiophilic substance of multilamellated appearance. Originally thought to derive from mitochondria (*38-41*), lamellar bodies are now considered to be synthesized from multivesicular bodies (*42, 43*). The type II cell is the only cell in the normal lung where these structures may be seen.

In 1954, Macklin (*44*) suggested that material may be secreted by the type II cell into the extracellular lining. Many electron micrographs of lamellar bodies, apparently in the process of being secreted, have since been published (*43, 45-49*). The secreted material is thought to be pulmonary surfactant and the lamellar bodies are considered to be storage sites of the surface-active phospholipids. Cytochemical studies have indicated the presence of phospholipids in lamellar bodies (*37, 50*), and with the advent of methods for the isolation of these structures from lung homogenates a high phospholipid content has been confirmed (*51-53*). The major phospholipid of lamellar bodies is dipalmitoyl lecithin, which is also the major surface-active phospholipid of the extracellular lining. The presence of lamellar bodies in cloned rat alveolar type II cells grown in culture is *prima facie* evidence for the biosynthesis of surfactant phospholipids by this epithelial cell (*54*).

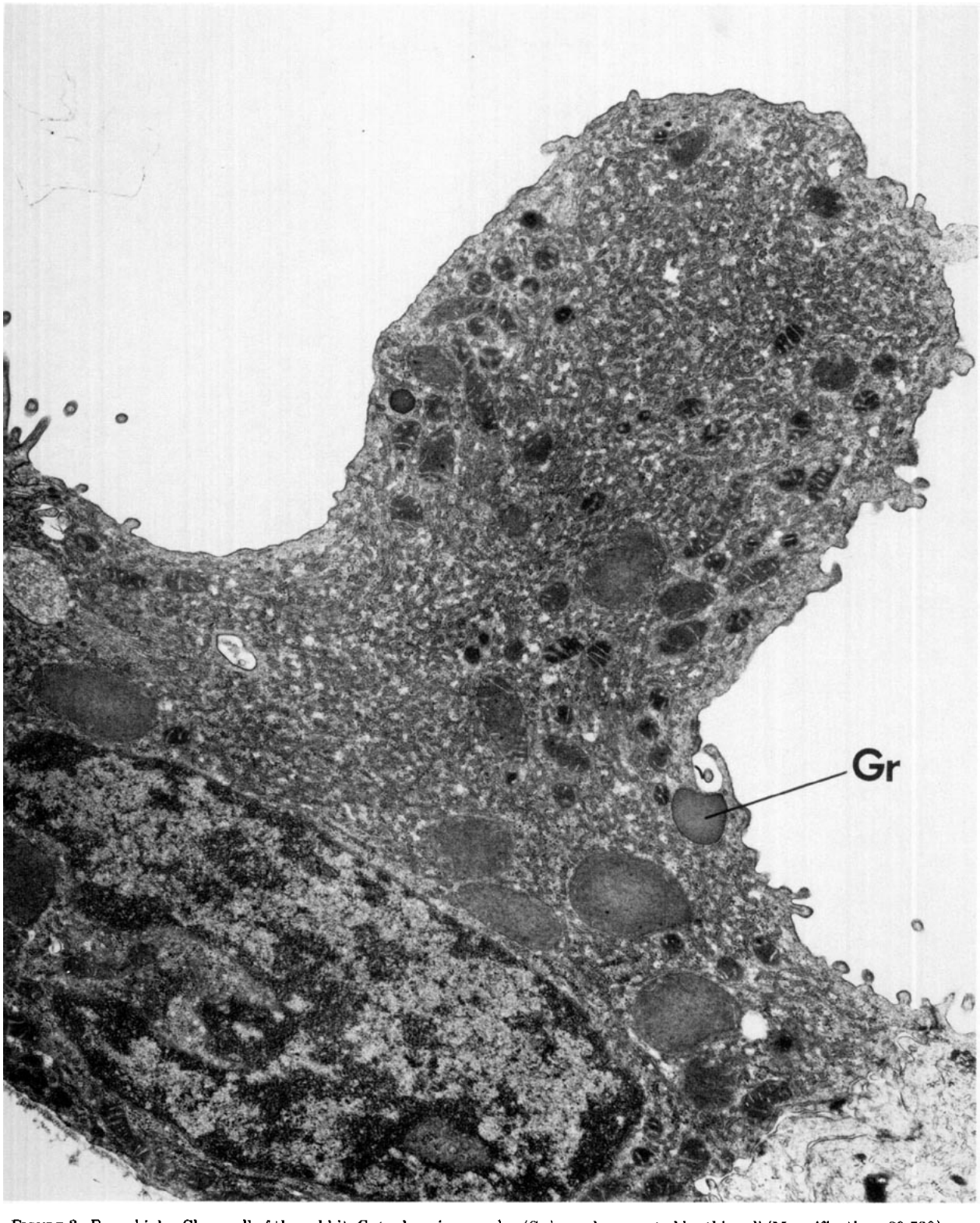

FIGURE 3. Bronchiolar Clara cell of the rabbit. Cytoplasmic granules (Gr) may be secreted by this cell (Magnification ×20,520).

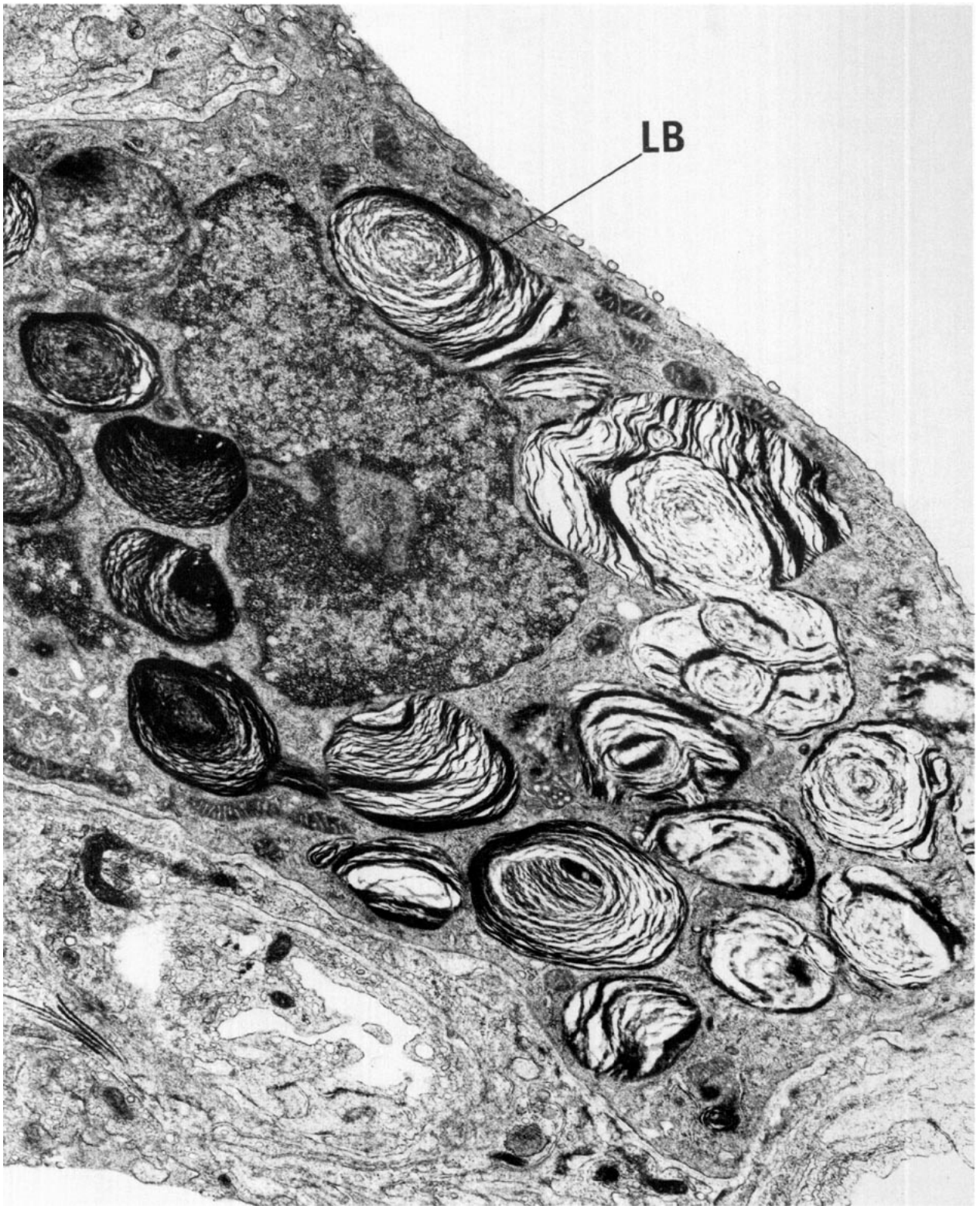

FIGURE 4. Alveolar type II cell of the rabbit. Lamellar bodies (LB) are storage sites of surface-active phospholipids. (Magnification ×20,520).

Other Cells

The emphasis placed here on the Clara cell and the alveolar type II cell should in no way distract from the important role many cells might have in elaborating important secretions for the respiratory tract. Beneath the pulmonary epithelium lie many other types of cells, such as mast cells, leukocytes, and plasma cells, which can respond to immunologic, inflammatory, or toxic stimuli.

Toxic Agents

The system, consisting of secretory cells and the extracellular lining, that we have briefly described is, of importance to the lung for such functions as gaseous diffusion, compliance, defense, and clearance. The location of this system is such that it should be vulnerable to the enormous variety of toxic agents ranging from particulate solids to gases. The surface area of the human respiratory tract is approximately 70 m^2, with a similar order of magnitude for the surface area of the underlying capillary bed. Thus, the lining system offers high accessibility to not only inhaled toxicants but also those which reach the lungs via the vascular circulation.

Many reagents are known to affect the lungs adversely but in most cases the target cells have never been elucidated. Interactions of toxic chemicals with secretory cells and the extracellular lining have been noted but few have been studied in detail. Interactions of particular importance are those which could produce alterations in the extracellular lining and hence impairment of pulmonary function.

Relationships between altered morphology of secretory cells and the functional capacity of the extracellular lining have not been established, although it may be assumed that degenerative alterations in the appearance of type II cells and perhaps Clara cells might affect their secretory abilities. Carbon dioxide, for instance, has been shown to produce hyalinelike membranes in animals with a concomitant depletion or vacuolization of the lamellar bodies (*55*). These effects, however, could be due to CO_2-induced acidemia. Also, alterations in the populations of these cells might affect the capacity for the formation, if not the quality, of the extracellular lining. Unfortunately the mechanisms which maintain the extracellular lining in dynamic equilibrium are not known and consequently toxic agents could affect those processes involved in removal of the lining as well as those synthetic and secretory aspects which we attributed to type II and Clara cells.

The recognition of target cells of toxic agents is extremely difficult when electron microscopy is the principal tool involved in the diagnosis, since cellular degeneration may not become apparent until events have progressed well beyond the initial lesion at the biochemical level.

The interaction of toxic chemicals with cellular constituents and morphological alterations are not necessarily confined to the same locus. The alterations in populations of a particular cell type may be initiated by toxic lesions produced in cell populations different from that observed to change. This appears to be especially true of the type II cells whose proliferation subsequent to type I cell injury is considered part of the normal repair process. For example, type II cells which are relatively resistant to the effects of the oxidant gases, oxygen, ozone, and nitrogen dioxide, nevertheless proliferate in response to injury inflicted upon the type I cell by these gases (*56-59*). Proliferative responses by the type II cell have been observed following exposure of animals to numerous toxicants such as cadmium (*60*), busulphan (*61*), bleomycin (*62*), heroin (*63*), silica (*64*), and urethane (*65*). However, with most toxic agents reasons for the proliferative response of the type II cell are not clear. Only the oxidant gases have been sufficiently well studied for it to be concluded with confidence that the type II cell responds to type I cell injury.

The proliferation of type II cells may also occur in the absence of apparent injury to type I cells. In radiation pneumonitis induced by inhalation of $^{239}PuO_2$ particles, type II cells appear highly sensitive to injury by α-radiation, whereas type I cells show signs of damage only in the terminal stages of the disease (*66*). The proliferation of type II cells seen in radiation pneumonitis may be a response to type II cell, rather than type I cell injury. Proliferation of type II cells in response to type II cell injury may be part of the repair process since other toxic agents such as busulphan (*61*) and bleomycin (*62*) cause type II cell damage as well as proliferation of this cell.

Proliferation of Clara-like cells in the bronchioles has been reported following exposure of animals to ozone (*67*) and nitrogen dioxide (*68*), and it is tempting to speculate that possible bronchiolar epithelial repair functions for the Clara cell exist which are similar to those defined for the alveolar type II cell. Clara cell proliferation has also been observed in response to other toxic compounds such as 4-nitroquinoline 1-oxide (*69*) and urethane (*70*), although with these particular compounds the alterations may be related to their carcinogenic activities: both of these com-

pounds can produce epithelial tumors in the lung. Clara cells shows signs of injury very soon after the injection of adrenalin (*71*). The type of injury, characterized by bleb or balloonlike structures at the apical regions of the cells, may not be uncommon with the Clara cell. The responses of Clara cells to adrenalin appear remarkably similar to processes which have been interpreted by others as apocrinic secretion. In fact, earlier studies of adrenalin-induced pulmonary edema (*72*) were interpreted to indicate enhanced secretion by Clara cells. However, as Wang, et al. (*71*) pointed out bleblike processes are not confined to Clara cells and similar effects may also be seen in type II cells and ciliated cells. Similar kinds of alterations in Clara cells are produced by inhalation of formaldehyde aerosols (*73*) and ammonia vapor (*74*).

Although many compounds appear to affect secretory cells of the peripheral airways, their effects on the extracellular lining are less well characterized. In most cases, alterations in the extracellular lining can only be implied from studies of electron micrographs. The manner in which the lining is affected and the extent to which its functions are impaired has received very little attention. Extreme reductions in pulmonary compliance associated with heroin-induced edema (*63*) and oxygen toxicity (*75*) and the appearance of hyaline membranes similar to those found in premature infants with respiratory distress syndrome suggest that the extracellular lining may have been severely altered although other mechanisms are also possible. Edema fluid which is often present in lungs exposed to toxic materials further complicates the issue, since edema fluid can affect the surfactant properties of the extracellular lining (*76*). However, absence of extracellular lining may also cause edema and, consequently, reasons for toxicant effects on the extracellular lining are difficult to determine.

From light and electron micrographs, the presence of unusual components in the peripheral airways generally indicates at least compositional changes in the extracellular lining. Proteinaceous materials in alveoli such as that seen following inhalation of aluminum dust (*77*) and silica (*64*) appear to be tolerated reasonably well, since respiratory distress does not accompany the induced lesions. Other toxic materials such as ozone (*78*), paraquat (*79*), and certain anesthestics (*80*) also appear to affect adversely the extracellular lining as studied by lavage.

Perhaps directly related to secretory cells and their secretions are the effects of a number of drugs which produce lipidosislike morphological alterations in the lung as well as other organs (*81*). The list of these drugs (*81*) includes a number of tricyclic antidepressants, antihistaminic, antihypercholesterolemic, and anorectic drugs. Common to all of these drugs is the production of lamellar structures similar to those found in type II cells, in a number of nonpulmonary tissues as well as some non-type II pulmonary cells, such as alveolar macrophages and Clara cells (*82, 83*). Chlorcyclizine appears to produce not only lamellar structures in cells not normally found to contain such structures but also hyperplasia and hypertrophy of the type II cell and the accumulation of lamellar body-related materials in the lumen of the alveoli. Reasons for these effects appear to be related to the amphipathic nature of drugs and their ability to associate with phospholipids. More precise reasons have not been elucidated, but it would appear that these compounds could be directly affecting lamellar body formation and/or secretion by the type II cell.

We have considered briefly the capacity of some toxic agents to affect the extracellular lining as well as those cells which may be responsible for many of its components. Reasons for the susceptibility of the alveolar regions of the lung to so many diverse toxic agents are not clear. Certainly, it seems unlikely that cellular processes unique to the lung offer specific sites for interactions with a vast number of toxicants. However, the extracellular lining could help explain the unusual susceptibility of alveolar cells to toxic agents were the lining directly involved in mediating the toxic effects. The extracellular lining being rich in phospholipids, may serve to store and concentrate lipophilic or amphipathic agents until sufficient quantities have accumulated to affect those metabolically highly active cells with which the lining is in intimate contact.

We wish to thank Linda B. Gilmore, Frederick A. Talley, and Drs. B. N. Gupta and E. E. McConnell for assistance in the preparation of the micrographs.

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